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Isotope ratio MS

LC-IRMS: Enhanced workflow for honey analysis using the new LC IsoLink II IRMS System

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Keywords

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Introduction

The advances in analytical instrumentation capabilities enables progress in methods and applications of LC-IRMS for food authentication purposes. The Thermo Scientific™ LC IsoLink™ II IRMS System features unique, patented technology and user-friendly design to ensure cost-efficient sample processing and high throughput. Laboratories investigating food fraud benefit from the system automation, preventive maintenance features and intuitive software. We demonstrate here how the LC IsoLink II IRMS System is designed to streamline honey fraud analysis and offer a reliable, robust tool for the investigation of isotope fingerprints.

Instrumentation setup

All measurements are performed using the LC IsoLink II IRMS System, consisting of the Thermo Scientific™ LC IsoLink™ II Conversion Interface that is fully integrated within the Thermo Scientific™ Vanquish™ Core HPLC system. A liquid sample is injected in mobile phase and all organic compounds eluting from the HPLC column are analyzed while maintaining the chromatographic resolution. Solutions of sodium persulfate and phosphoric acid are mixed 1:1 using the proportioning valve of the Vanquish Core Dual Pump C (modified) at a combined flow rate of 0.1 mL/min. The HPLC eluate is added to this highly oxidative mixture in a high-pressure mixing-T and is subsequently transported into the conversion reactor. Here, the individual LC fractions are quantitatively oxidized within the aqueous solution, and the resulting CO_2 is removed from the liquid phase in a downstream degassing unit and entrained into a stream of He for admission to the Thermo Scientific™ DELTA™ Q IRMS via a Thermo Scientific™ ConFlo IV™ Universal Interface (Figure 1). Full LC IsoLink II IRMS System operation is driven by the Thermo Scientific™ Qtegra™ ISDS Software that features integration with Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) Software capabilities.

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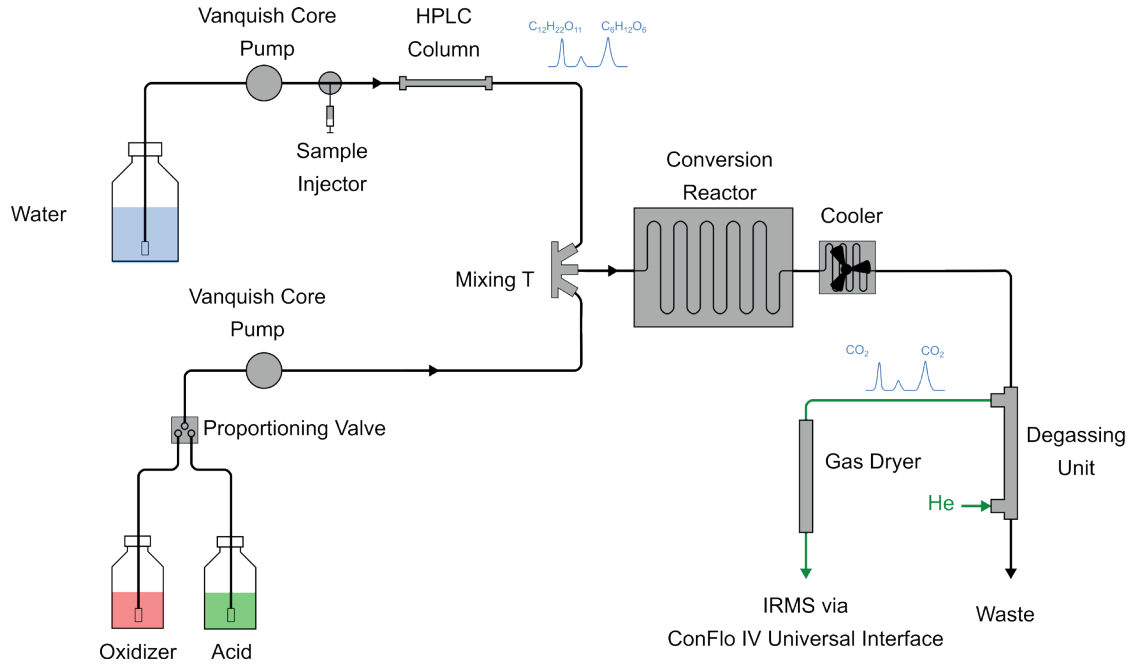

Figure 1. Simplified LC IsoLink II IRMS System workflow

Patented backflush system for preventive maintenance

The high-throughput analysis of honey requires extraordinary robustness of the LC-IRMS system. To fulfill this high demand, the LC IsoLink II Conversion Interface comes with a novel backflush functionality, providing an automated cleaning procedure for the degassing unit and the conversion reactor.

When in backflush mode, the eluent is passed through the reactor in reverse, thereby removing any particles which have accumulated at the inlet of the reactor or in the degassing unit (Figure 2). Regular employment of the backflush feature minimizes flow path blockage and maximizes system uptime and productivity.

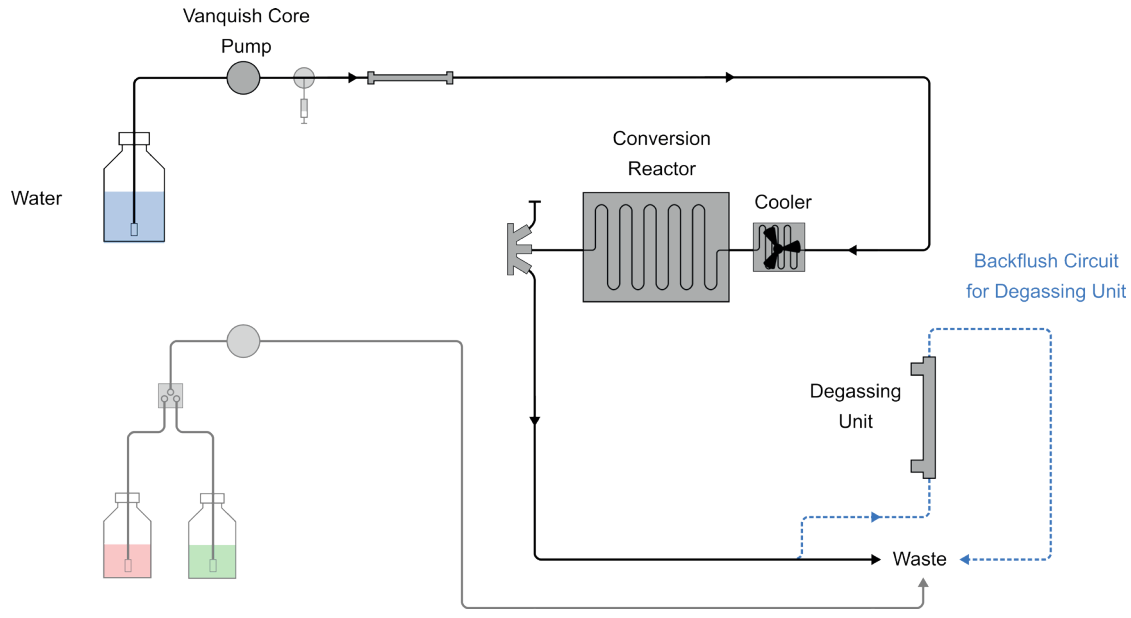

Figure 2. LC IsoLink II Conversion Interface backflush functionality for preventive maintenance

The modular pull-out design of LC IsoLink II Conversion Interface guarantees easy accessibility to all serviceable components that are placed in a drawer (Figure 3), allowing quick maintenance. The conversion reactor comes in a novel cartridge-based design that can be exchanged by users within minutes, if required.

Figure 3. LC IsoLink II Conversion Interface pull-out drawer for easy access to all system components

The LC IsoLink II IRMS System employs the state-of-the-art, electronically regulated Vanquish Core Dual Pump C for both eluent and reagent supply. This provides stable, flat baselines (Figure 4), benefitting both the peak integration and working range of the LC-IRMS measurements.

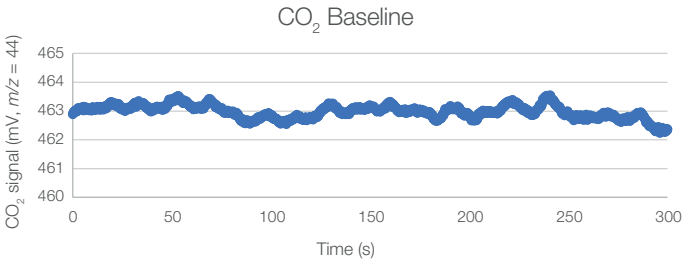

Figure 4: Typical baseline CO_2 signal of the LC IsoLink II IRMS System setup for honey analysis

The Vanquish Core Dual Pump C is fully controlled via Qtegra ISDS Software, allowing on-the-fly pressure trace monitoring and automated adjustment of liquid flows, e.g. in case of leaks.

ConFlo IV Universal Interface integration

The integration of the ConFlo IV Universal Interface in the LC IsoLink II IRMS System allows for automatic stability and linearity checks and corrections, automatic reference dilution and software-controlled switching between multiple preparation devices. All functionalities of the ConFlo IV Universal Interface are controlled by the Qtegra ISDS Software. Automated precision and linearity checks over an extended period of honey samples analysis (Table 1) report high stability of $\delta^{13}C$ values across the range of signal amplitude observed for typical LC-IRMS honey analysis. In a case of larger deviations in linearity values, Qtegra ISDS Software offers automated linearity corrections for measured data.

Day	Precision 1 σ (‰)	Linearity (‰/nA)
1	0.031	**0.018**
10	0.033	**0.015**
20	0.053	**0.006**
30	0.045	**0.011**

Table 1. DELTA Q IRMS $\delta^{13}C$ precision and linearity (via reference gas pulses) during honey analysis

Analytical setup

Honey samples were prepared by dissolving 1 g of honey in 4.1 mL water and shaken until fully dissolved. The solution is diluted 1:100 and filtered through a 0.45 µm syringe filter directly into an autosampler vial. After preparation, the vials are kept in the autosampler at 10 °C to avoid microbial degradation of the samples. Separation of the sugar fractions is carried out using an isocratic HPLC method with water as an eluent (Table 2) that is set up through the Thermo Scientific™ ChromControl Plug-In in the Qtegra ISDS Software.

Column	
Type	Shodex™ Sugar SC-1011
Operating temperature	60 °C
Injection volume	10 µL
Total runtime	40 min
Eluent	
Type	H_2O
Flow	0.3 mL/min
Pressure	15 – 18 bar
Reagents	
Type	$Na_2S_2O_8$ (0.3 mol L^{-1}), H_3PO_4 (8 %)
Flow	0.1 mL/min, 50:50

Table 2. LC IsoLink II IRMS setup for honey analysis

A typical honey sample analysis timeline is shown in Figure 5, resulting in ca. 40 min analysis time. The Qtegra ISDS Software LabBook displays the key method parameters in a simple Drag&Drop layout, enabling easy measurement setup.

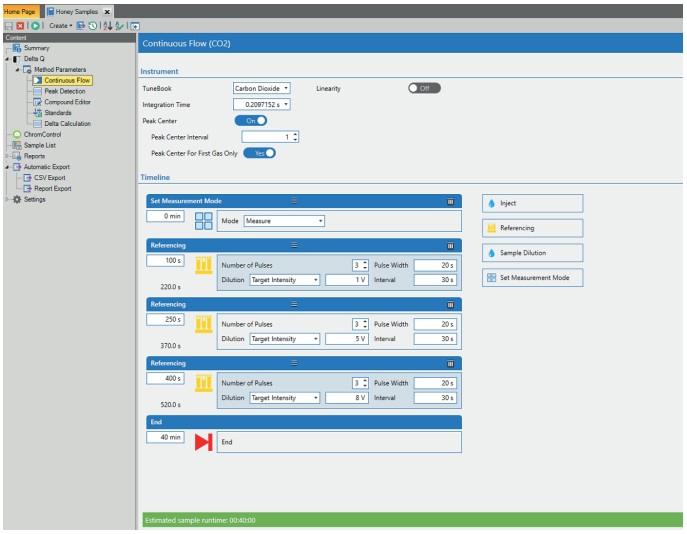

Figure 5. Qtegra ISDS Software panel for methods parameter setup

To ensure quality control of measurements and data normalization, honey samples are analyzed together with glucose standards (Figure 6). Within a LabBook, a single sample list is created (Figure 6) that controls the entire LC-IRMS system, including the HPLC components and the IRMS data acquisition. The LabBook includes everything surrounding the sample analysis: the method, the data evaluation, the results and the QC. Qtegra ISDS Software provides integrated data reduction and correction options, including compound specific isotope analysis standards. Quality control options allow continuous quality control checks to prevent loss of samples and ensure the high quality of your data.

Sample List

	Label	Status	Sample Type	Comment	Vial	Reference	Inject Volume [µl]	Inject
1	Glucose Std 01-1		Delta Standard (CSIA)	Glucose Batch 25	R:E1	Glucose	10	☑
2	QC Honey 01-1		QC Standard	QC Batch 12	R:A1	QC Honey	10	☑
3	QC Honey 01-2		QC Standard	QC Batch 12	R:A1	QC Honey	10	☑
4	QC Honey 02-1		QC Standard	QC Batch 12	R:A2	QC Honey	10	☑
5	QC Honey 02-2		QC Standard	QC Batch 12	R:A2	QC Honey	10	☑
6	Honey 01-2		Unknown	<Comment>	R:A3		10	☑
7	Honey 02-1		Unknown	<Comment>	R:A3		10	☑
8	Honey 02-2		Unknown	<Comment>	R:A4		10	☑
9	Honey 03-1		Unknown	<Comment>	R:A4		10	☑
10	Honey 03-2		Unknown	<Comment>	R:A5		10	☑
11	Honey 04-1		Unknown	<Comment>	R:A5		10	☑
12	Honey 04-2		Unknown	<Comment>	R:A6		10	☑

Figure 6. Exemplary Qtegra ISDS Software's sample list for honey analysis

Qtegra ISDS Software ensures the utmost integrity and traceability for raw data, metadata, and reported results. The software security supports the administrator by creating a controlled environment to ensure that the system is configured to comply with GxP and 21 CFR Part 11 regulations. Qtegra ISDS Software enables audit trails, access privileges, password protection, electronic signatures and more to meet user's compliance goals. Additionally, full integration with LIMS is supported. Sample lists can be imported from LIMS into pre-defined protocol templates, incorporating the full range of quality control checks. Comprehensive, user-definable reports allow for flexible export to external LIMS software packages.

Results

For evaluating robustness and reliability of the new LC IsoLink II IRMS System we performed extensive honey analysis on three serial systems. All three LC IsoLink II IRMS Systems were running honey analysis over a period of ca. 3 months, with a minimum of 1500 samples analyzed on each, including control standards. All systems utilized the newly developed cartridge-based conversion reactor and backflush functionality for a daily automated cleaning procedure that resulted in stable and reliable system performance (Figure 7).

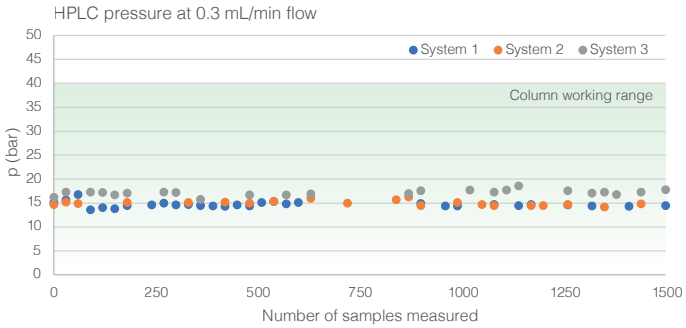

Figure 7. Robustness test of three LC IsoLink II IRMS Systems demonstrating high stability of the HPLC pressure, without system downtime

Typical separation of the sugar fractions is shown in Figure 8. Qtegra ISDS Software supports easy labeling of chromatograms using the softwares Click&Identify approach, allowing users to build a global compounds library for quick peak identification and data organization. The data can be queried over multiple LabBooks simultaneously to assess the long term reproducibility of quality control results.

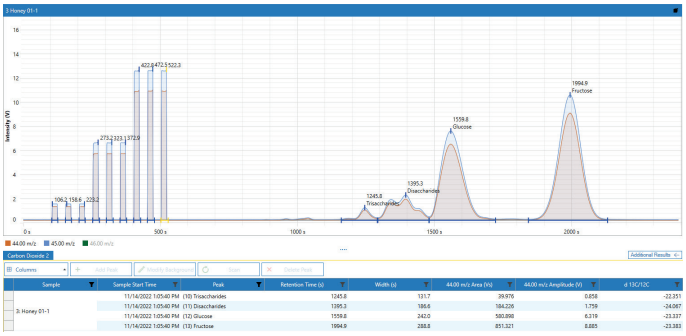

Figure 8. LC-IRMS honey chromatogram with separated glucose, fructose, disaccharides and trisaccharides fractions, including ConFlo IV Universal Interface automated reference gas injections

Figure 9 shows excellent repeatability over 581 measurements of acacia honey, the QC laboratory standard, demonstrating high robustness and reliability of the LC IsoLink II IRMS System. The standard deviation of all measurements was below 0.14 ‰, including the low concentrated trisaccharides fraction (Table 3).

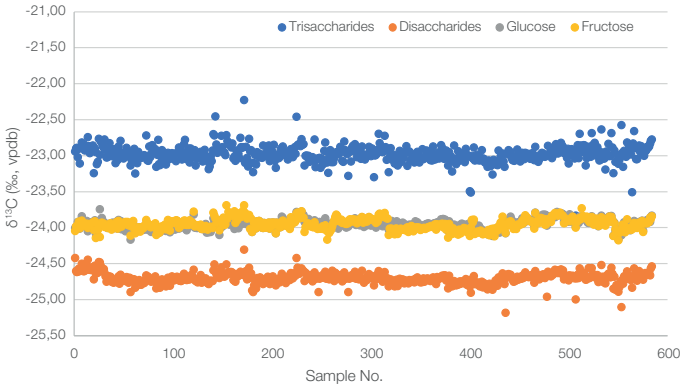

Figure 9. $\delta^{13}C$ during long-term robustness measurements of QC honey standard using the LC IsoLink II IRMS System

n=581	Trisaccharides	Disaccharides	Glucose	Fructose
$\delta^{13}C$ average (‰)	-22.98	**-24.70**	**-23.95**	**-23.95**
SD	0.13	**0.08**	**0.06**	**0.08**

Table 3. High precision of 581 honey measurements (QC laboratory standard) using the LC IsoLink II IRMS System

Conclusions

LC-IRMS of honey samples is a quantitative and fractionation-free methodology that is globally accepted and used for routine control for approximately 15 years. With the next generation LC IsoLink II IRMS System we have significantly improved the robustness and reliability of the LC-IRMS analysis, ensuring high efficiency and data quality for the analytical laboratories running honey analysis and beyond. The patented backflush functionality, the new reactor design and the system automation driven by Qtegra ISDS Software provide cost-efficient and streamlined analysis with high precision results.

Learn more at **thermofisher.com/lc-isolink**